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Application of stable isotope ratio analysis for biodegradation monitoring in groundwater

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Stable isotope ratio analysis is increasingly being applied as a tool to detect, understand, and quantify biodegradation of organic and inorganic contaminants in groundwater. An important feature of this approach is that it allows degradative losses of contaminants to be distinguished from those caused by non-destructive processes such as dilution, dispersion, and sorption. Recent advances in analytical techniques, and new approaches for interpreting stable isotope data, have expanded the utility of this method while also exposing complications and ambiguities that must be considered in data interpretations. Isotopic analyses of multiple elements in a compound, and multiple compounds in the environment, are being used to distinguish biodegradative pathways by their characteristic isotope effects. Numerical models of contaminant transport, degradation pathways, and isotopic composition are improving quantitative estimates of *in situ* contaminant degradation rates under realistic environmental conditions.

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Introduction

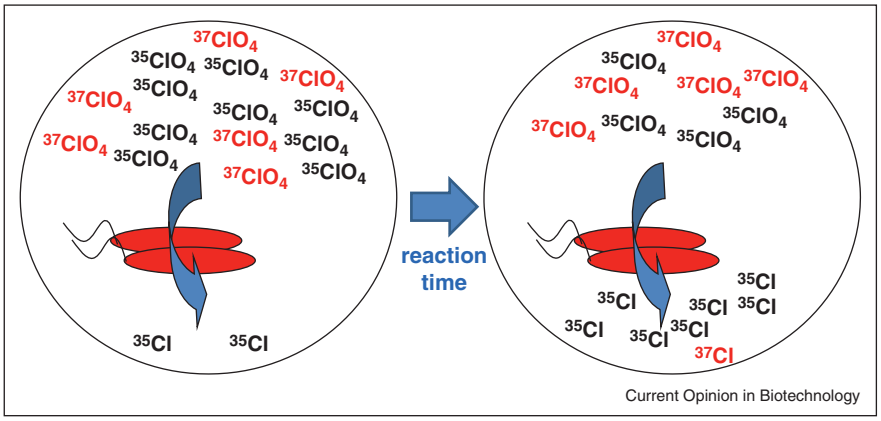
Biodegradation of anthropogenic chemicals in the environment has been a subject of active research for several decades. Some of the earliest studies were motivated by a need to understand the fate of organic pesticides brought into widespread use after World War II [1]. Since that time, biodegradation research has expanded to encompass nearly every class of organic and inorganic compound used by humans, from fuels and solvents to agricultural chemicals and common household drugs, as many of these compounds, as well as precursors used in their production, ultimately end up in soils and groundwater. Biodegradation research has resulted in the development of a wide variety of different field approaches to monitor intrinsic contaminant biodegradation ('monitored natural attenuation'; MNA; [2]) and to enhance its progress – a practice now known as 'bioremediation'. One of the primary difficulties encountered during MNA assessments and when determining the effectiveness of *in situ* bioremediation techniques is distinguishing concentration changes caused by degradative losses from those caused by abiotic processes such as dilution, dispersion, volatilization, and sorption. Natural heterogeneities in aquifer systems and in contaminant distribution and transport can complicate interpretations; conclusions concerning chemical fate must often be based on data from a small set of groundwater samples, taken from a limited number of broadly screened monitoring wells.

Stable isotope ratio measurements of specific compounds can be used to distinguish biodegradation from non-destructive processes affecting concentration. Isotope effects can also be used to constrain reaction progress and, in concert with spatial/temporal data, to estimate reaction rates. Moreover, in recent years, attempts have been made to use stable isotope fractionation data to discriminate different reaction pathways of contaminant degradation in groundwater. This paper reviews selected recent developments (with emphasis on the last few years) in the application of stable isotope ratio analysis for biodegradation monitoring in aquifers and discusses some of the limitations of this approach.

Fractionation of stable isotopes

The utility of stable isotopes for assessing biodegradation is based on the fact that chemical bonds formed by a heavy isotope of an element typically are stronger than those formed by a light isotope of the same element in the same compound. Thus, when a specific bond is broken in a compound, molecules containing heavy isotopes of elements involved in the bond (or adjacent to it to a lesser extent) generally react more slowly than molecules containing light isotopes of those elements [3]. As a result, the heavy isotopes usually become enriched in the residual reactant compound and depleted in the product (Figure 1). This process is known as isotopic fractionation and, when it is associated with a unidirectional reaction (rather than a reversible phase change or other equilibrium process), as a kinetic isotope effect. In simple systems, such effects can be described by the Rayleigh equation (Eq. (1)), which relates the concentration of a

Figure 1



Schematic illustration of kinetic isotopic fractionation during biodegradation of perchlorate, causing relative enrichment of a heavy isotope in the reactant (^{37}Cl in ClO_4^-) and its depletion in the product (^{37}Cl in Cl^-). Overall reduction of ClO_4^- to Cl^- proceeds visually from top to bottom through the action of bacteria (red shapes).

chemical to its isotopic enrichment during a reaction such as biodegradation, with the relative isotopic discrimination of the reaction given by the epsilon value (ε) (Eq. (2))

$$R = R_o f^{(\alpha-1)} \quad (1)$$

$$\varepsilon = \alpha - 1 \quad (2)$$

where R = molar ratio of the heavy to the light isotopes (e.g. $^{13}C/^{12}C$) in the reactant, R_o = initial molar ratio of the heavy to light isotopes in the reactant, f = fraction remaining of the initial reactant consisting of the light isotope, or denominator in R (e.g. $^{12}C/^{12}C_o$, typically approximated by the fraction of total reactant compound remaining, C/C_o), α = isotope fractionation factor, and ε = enrichment factor. Isotope fractionation effects are small (ε values commonly are reported in parts per thousand, or ‰) and must, therefore, be determined with high precision. Isotope ratios of samples typically are measured and reported as differences from the ratios in common standards, for example:

$$\delta^{13}C = R_{sample}/R_{standard} - 1, \quad (3)$$

with uncertainties on the order of ±0.01 to 1‰, depending on the element and the method of analysis.

The Rayleigh equation, originally developed to describe fractionation of binary gases during distillation [4], is commonly applied to isotope data to quantify many different processes, including biodegradation, because it directly relates changes in reaction progress (f) to bulk isotopic composition (R) of the reactant, with the isotopic enrichment factor (ε) as a potential indicator of the reaction mechanism. An example of the application of this equation for describing the fractionation of O and Cl isotopes during perchlorate biodegradation is given in Figure 2 [5]. The Rayleigh equation can be re-arranged to give the isotopic composition of the instantaneous or cumulative reaction product at any point along the reaction path. Although this simple approach is commonly used, it has a number of limitations, and more sophisticated modeling approaches may be necessary to interpret isotope data for complex reactions and heterogeneous environmental conditions.

Applications to biodegradation

The most direct and common application of stable isotope analysis for contaminant fate monitoring is to identify the occurrence of biodegradation based on changes in a single isotope ratio (e.g. $^{13}C/^{12}C$) or multiple isotope ratios (e.g. $^{13}C/^{12}C$ and $^{2}H/^{1}H$ or $^{37}Cl/^{35}Cl$) in a compound. Where a contaminant exhibits spatial or temporal variations in concentration, the isotope ratios may indicate whether this is caused by biodegradation or by other processes with different patterns of isotopic variation (e.g. source changes) or by non-fractionating processes (e.g. dilution). In favorable circumstances, the magnitude of isotope effects (variations in R) can be related to the progress of the biodegradation reaction where concentration changes alone may be difficult to interpret. The isotopic approach has been used to document degradation of many common organic groundwater contaminants, including chlorinated solvents [6,7,8•] and key degradation intermediates of these solvents [8•,9•,10], typical gasoline components (e.g. benzene, toluene, xylenes, ethylbenzene) [8•,11], and gasoline additives such as MTBE [12] and 1,2-dibromoethane [13], as well as inorganic contaminants such as nitrate, chromate, selenate, and perchlorate [14–18]. The term ‘compound-specific isotope analysis (CSIA)’, which appears commonly in isotopic studies of organic contaminants performed by the GC-IRMS method, is used here more generally to include isotope-ratio measurements on specific organic and inorganic compounds by various